Texas A&M University at Galveston
1001 Texas Clipper Road
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ELEVENTH INTERIM PERFORMANCE REPORT

FEBRUARY 29TH, 2024

Project Title: The Fate and Toxicity of Microplastics and Persistent Pollutants in the Shellfish and Fish of Matagorda Bay

Submitted To:

Matagorda Bay Mitigation Trust

Performing Laboratory:

Texas A&M University on behalf of Texas A&M University at Galveston

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The Fate and Toxicity of Microplastics and Persistent Pollutants in the Shellfish and Fish of Matagorda Bay

Personnel

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Mr. Steven J. Raabe

Location(s):

Texas A&M University at Galveston

Project Duration:

01 June 2021 – 31 August 2024

Objectives:

Objective 1: Quantify the extent of microplastics pollution in the surface waters and biota of Matagorda Bay.

Objective 2: Measure levels of persistent pollutants in surface waters, adsorbed to microplastics, and bioaccumulated in the biota of Matagorda Bay.

Objective 3: Study the toxicity of microplastics and adsorbed pollutants using embryo-larval life stages of sheepshead minnow.

Objective 4: Public educational outreach to local high school students on the science of ecosystem health monitoring.

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1. INTRODUCTION

1.1 Background

This project is studying the extent of microplastics, and persistent pollutant exposure of resident biota (shellfish and fish) sampled from Matagorda Bay and assessing any likely toxicity effects due to exposure. The *new knowledge* gained from the successful completion of this project will contribute to an understanding of the long-term fate and toxicity of microplastics (and adsorbed pollutants) in the Matagorda Bay system.

In this eleventh interim report (December 1st, 2023 – February 29th, 2024) we provide a list of key accomplishments as per the third quarter of Year 3 of the project.

2. Key Updates

As of the period encompassing the eleventh interim report (December 1st, 2023 – February 29th, 2024), the key achievements associated with each stated objective are detailed below.

Objective 1: Quantify the extent of microplastics pollution in the surface waters and biota of Matagorda Bay.

- We have completed all analysis of microplastics in the body-burdens (muscle and liver) of biota from Matagorda Bay.
- We are currently preparing manuscripts for intended submission in Spring 2024.

Objective 2: Measure levels of persistent pollutants in surface waters, adsorbed to microplastics, and bioaccumulated in the biota of Matagorda Bay.

- We have completed all analyses of PAHs and PCBs in the body-burdens (muscle and liver) of biota from Matagorda Bay.
- Our current focus is on preparing a high-impact manuscript for intended submission in Spring 2024.

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Objective 3: Study the toxicity of microplastics and adsorbed pollutants using embryo-larval life stages of sheepshead minnow.

- We are currently performing single or mixtures toxicity studies with select PAHs, PCBs, and microplastics.
- The toxicological study design involves exposing two days post fertilized (dpf) zebrafish (*Danio rerio*) embryos to select pollutants or their mixtures for up to 4 days (96 hours) or up to 6 dpf. Fish are exposed under a semi-static renewal exposure design which involves replacing 50% of the exposure aquaria for each treatment group for each day of the trial. Up to 25 embryo/larval fish are exposed to each test chemical. At test termination, n=10 fish are placed in a multi-well micro-respirometer plate to measure oxygen consumption (or metabolic rate) over a duration of several hours (≤6 hours). Another subset of n=5 fish is observed under a microscope to quantify key morphological features, such as fish size, shape, presence of anomalous features, etc.). The final sub-set of n=10 fish is placed on a solution containing a chromogenic substrate that changes color to reflect the metabolic biotransformation capacity of the fish.
- The single compound toxicity trials include exposure to select PAHs and PCBs, which were chosen based upon their abundance and detection frequency in the biota sampled from Matagorda Bay. The shortlisted PAHs (and their concentrations) to be tested as single compounds include: phenanthrene (0.2 µM), pyrene (0.1 µM), Benzo(a)anthracene (0.6 µM), and indeno[1,2,3-cd]pyrene (0.1 µM). And the shortlisted PCBs (and their concentrations) to be tested as single compounds include: PCBs 18 (0.1 µM), 81 (0.02 µM), 105 (0.04 µM), and 0.02 (µM). We are using polystyrene (PS) latex beads (0.1 µm) as a representative microplastic at a final concentration of 10 µg/L.
- We will also perform mixtures toxicity trials in which each single compound (i.e., PAH or PCB) will be combined with the PS microplastic (10 µg/L). In addition to these tests, the PAHs or PCBs will be combined as separate PAHs only or PCBs only mixtures, PAHs only mixture + PS, PCBs only mixture + PS, or PAHs + PCBs + PS mixture.
- In **Fig. 1** we show preliminary data of our first trial with the PAH, phenanthrene (PHE, 0.2 µM). The microplastic polystyrene (PS) was tested at a single concentration of

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10 µg/L. And the two pollutants were tested as a mixture of PHE (0.2 µM) + PS (10 µg/L). In addition, we also tested a solvent control group comprising 0.1% $^{v}/_{v}$ dimethyl sulfoxide (DMSO). The use of a carrier solvent was necessary to dissolve or solubilize the hydrophobic PAH.

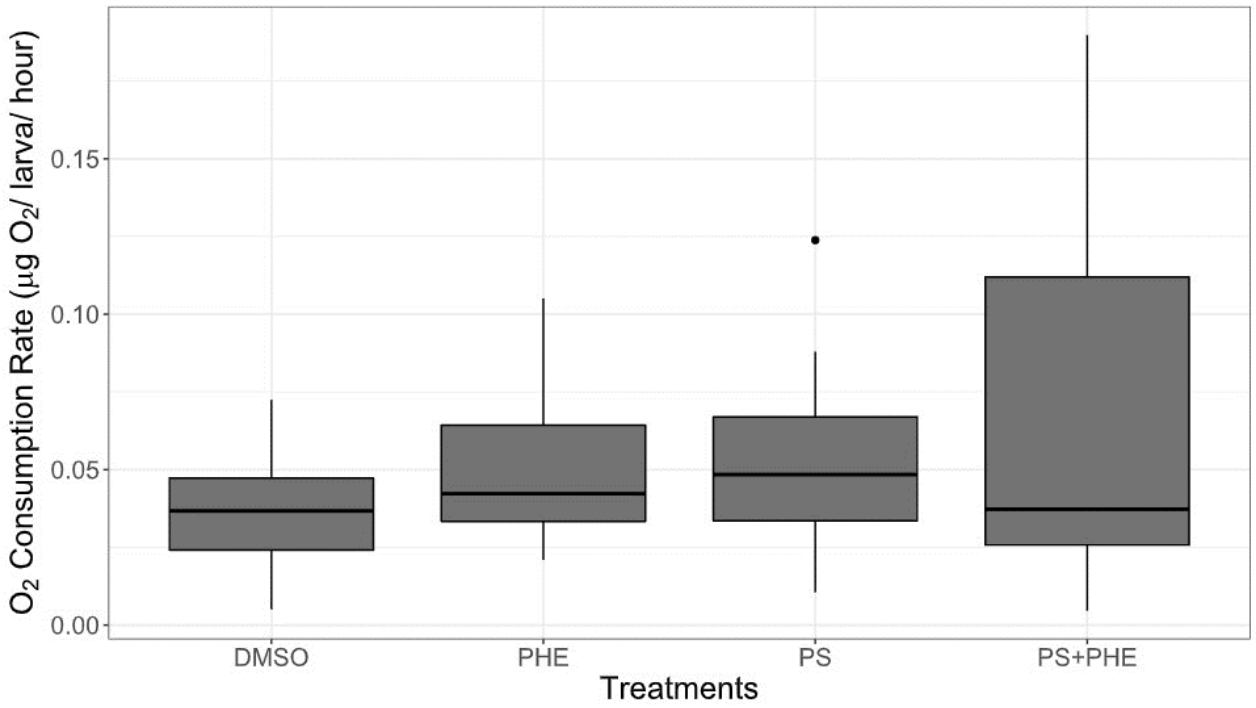

Fig. 1. The effects of exposure of ~6 dpf embryo/larval zebrafish metabolic rate (or oxygen consumption rate) to single test compounds, PHE = phenanthrene (0.2 µM), and PS = 0.1 µm polystyrene latex beads at 10 µg/L; or their mixture PS+PHE = phenanthrene (0.2 µM) + polystyrene (10 µg/L). A solvent control group (0.1% $^{v}/_{v}$ DMSO) was also tested. No statistically significant effects of exposure was observed.

- While no statistically significant effects of exposure was evident for the single compounds or mixture vs. the solvent control group, the mixtures exposed group (i.e., PS+PHE) exhibited an increased coefficient of variation of 28 – 49% higher than the remainder groups. Therefore, while the mean or median values are not appreciably different betwene

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the treatment groups, the mixtures group appears to exhibit considerable variation in metabolic rate, likely indicating an increased stress response in the exposed fish.

- We are also characterizing the effects of exposure on the cardiac morphology of larval fish (**Fig. 2**) and expect to report on our findings by the next interim report.

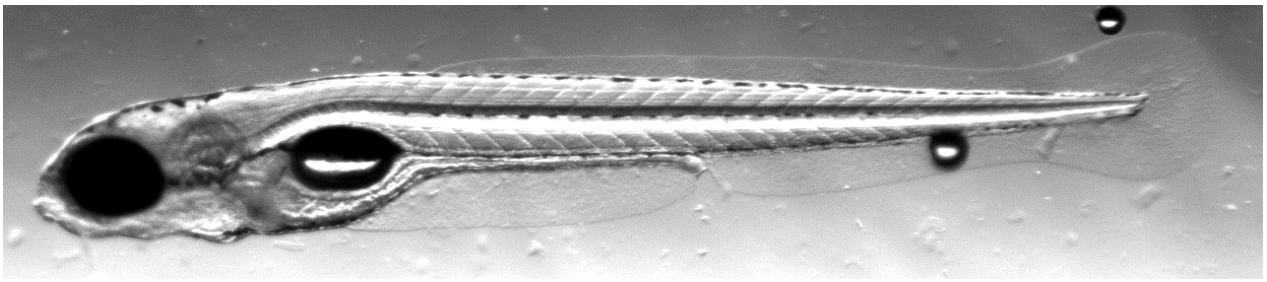

Fig. 2. An image of a 6 dpf larval zebrafish. Image analysis will be used to assess the effects of exposure on morphology of fish.

Objective 4: Public educational outreach to local high school students on the science of ecosystem health monitoring.

- Educational outreach engagement was pursued in collaboration with the TAMUG Sea Camp program in Summer 2022. Outcomes from the outreach activity were reported in the *sixth* interim report.

3. FURTHER WORK

Planned work for completion over the duration of the *twelfth* interim report (Year 3) are as follows:

1) Prepare and submit manuscripts for publication by Summer 2024 on PAHs, PCBs, and microplastics levels in biota from Matagorda Bay.

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2) Prepare a manuscript describing the microplastics analysis methods and application to measuring levels in biota from Matagorda Bay (Spring 2024).
3) Complete toxicological studies on the effects of microplastics and PAH/PCB single compounds or mixtures on embryo-larval life stages of fish (complete by summer 2024).

4. REFERENCES

None reported for this interim report.

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Reviewed by:

2/28/2024

_____ Date: _____

Dr. David Hala, TAMUG, P.I.

Approved by:

_____ Date: 2/29/2024

Mr. Steven J. Raabe, Trustee